EMMA ID: *EM:15539*
Gene: *Gpx1, Cdh23*
Common name: *GPx1ko-Cast*
Allele: $Gpx1^{tm1.1(KOMP)Vlcg,}Cdh23^{Ahl+}$

Allele Information

The gene coding the cellular glutathione peroxidase 1 (Gpx1) has been silenced by KOMP as described elsewhere (https://www.mmrrc.org/catalog/sds.php?mmrrc_id=47982). Mice were backcrossed to the C57Bl/6.CAST-Cdh23Ahl+ background in the laboratory of Prof. Georg Klump at the Unviversity of Oldenburg.

Links to the general information

About IKMC resource https://www.infrafrontier.eu/knowledgebase/protocols/ikmc-products

IMPC mouse phenotype data, search by the gene name
http://www.mousephenotype.org/

Genotyping Information

Genotyping by end-point PCR based on gel is composed of a genespecific short range PCR using primers on wild type allele and a mutant allele-specific short range PCR. The combined results show the genotype of the mice. For example: mutant positive, wild type positive = Heterozygous.

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wild type	Gpx1-F	Gpx1-R	424
Mutant	Gpx1-F	GT Gpx1- F	270

Primer sequences

Primer Name	Sequence 5' --> 3'
Gpx1-F	CAGCCGGAAAGAAAGCGATG
Gpx1-R	AGAAAGTAAGCGGTGTCCCG
GT Gpx1- F	CAGCCGGAAAGAAAGCGATG
Polymerase	All Taq

PCR setup (AllTaq)

Component	Volume (µl) 1x	Final conc.
DNA (~ 50-100 ng)	2	
Q-Solution (5x)	2,5	0,5
PCR-Buffer (5x)	5	1
DNTP mix (10 mM)	0,5	0,2
$MgCl_2$ (25 mM)	1,5	1,5
Primer 1 (10 pmol/µl)	1	0,4
Primer 2 (10 pmol/µl)	1	0,4
Primer 3 (10 pmol/µl)	1	0,4
Taq Polymerase (5 U/µl)	0,5	2,5 U/rxn
H_2O*	10	
Final volume	25	

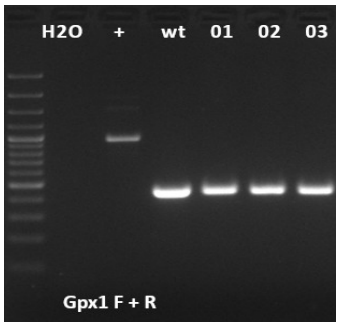
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

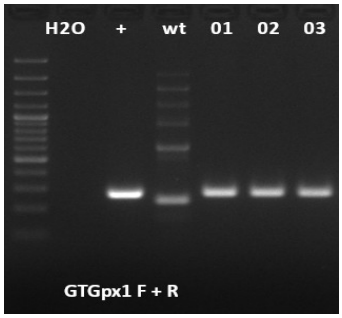
PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 60°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

Touch-Down cycling protocol: first 10 cycles anneal at 65°C, decreasing 1°C per cycle, next 30 cycles aneal at 55°C.These PCR conditions have been optimized for our methods and

Gel Image



WT-PCR



Mutant-PCR

+ het control

Separated by gel electrophoresis on a 2% agarose gel.